

## Gradual Evolution of Virulence of *Puccinia coronata* on Oats

M. D. Simons and M. E. McDaniel

ARS, U.S. Department of Agriculture, Department of Plant Pathology, Iowa State University, Ames 50011, and Department of Soil and Crop Sciences, Texas A & M University, College Station 77843, respectively.

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### ABSTRACT

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Seedlings of *Avena sativa* 'TAM 0-301' in the greenhouse at a mean temperature of 23 C were highly resistant to all isolates of *Puccinia coronata* from Texas that were tested in 1974. In 1975-1976, TAM 0-301 was moderately resistant to a few isolates; in 1977-1979 it was moderately resistant to a greater proportion of isolates and susceptible to some isolates. In 1980, TAM 0-301 was resistant to only 17% of all isolates, moderately resistant to 64%, and susceptible to 19%. In growth chambers, susceptibility

and high resistance, as seen in the greenhouse, were not temperature sensitive, but moderate resistance was. Isolates that induced moderately resistant reactions in the greenhouse generally induced highly resistant reactions at 20 C and susceptible reactions at 26 C. The same gene in TAM 0-301 conditioned both high resistance to some isolates and moderate, temperature-sensitive resistance to others.

The origin and spread of pathogenic forms of the cereal rust fungi having new virulence is a matter of great theoretical and practical concern to plant pathologists. In the past, such new forms have appeared suddenly in the rust population as the result of a major change in a single locus, with the new form differing sharply in virulence from previously detected forms. This phenomenon has been reported many times and was well exemplified by the sudden appearance in 1957 of race 290 of *Puccinia coronata* Cda. on oat cultivars having the Landhafer gene for resistance (9). This gene conferred a high degree of resistance to all forms of *P. coronata* previously known in North America; race 290 was fully virulent on oats having the gene. The appearance of new races does not always follow this pattern, however. Caldwell (1) speculated that wheat cultivars known to carry polygenic resistance to *Puccinia recondita* Rob. ex Desm. gradually lost their effectiveness because of gradually increasing virulence in the rust population. This might be expected where polygenic resistance is involved, but mutations involving lesser degrees of changing virulence also have been reported where the resistance of the host is known to be controlled by one or a few genes. Zimmer and Schafer (12) reported a spontaneous change in virulence of an isolate of *P. coronata* race 202 on the oat cultivar Ascençao in which a reaction of flecking without sporulation changed to one of small sporulating uredia surrounded by necrosis.

Watson and Luig (11) showed that *Puccinia graminis* Pers. could increase its virulence progressively on wheat cultivars known to have only a single resistance gene. The gene *Sr6* originally conditioned reactions of high resistance at low temperatures and of moderate susceptibility at high temperatures in Australia. Forms of *P. graminis* showing at least three distinct levels of virulence then appeared, with the most virulent inducing moderately susceptible reactions at both low and high temperatures. Four forms showing a range of virulence on wheat cultivars with the *Sr11* gene, which is not temperature sensitive, were demonstrated experimentally. A gradual increase in average virulence of isolates of *P. coronata* in small imperceptible steps over a period of years on the differential oat cultivars Victoria and Trispermia was reported by Michel and Simons (6).

In the early 1970s, the oat cultivars TAM 0-301 and TAM 0-312 were developed and released for use in southern Texas where crown

rust is often severe. They were grown extensively in that area during the period of this study. TAM 0-301 possesses the resistance gene *Pc-58* and TAM 0-312 possesses *Pc-59*, both from strains of *Avena sterilis* introduced from the Middle East (3,4,10). When these two cultivars were released, they were highly resistant to the forms of *P. coronata* occurring in Texas, but within 1-2 yr there was some evidence that their resistance was beginning to lose its effectiveness. This study was undertaken to document the appearance and spread of forms *P. coronata* virulent on oats with genes *Pc-58* and *Pc-59*.

### MATERIALS AND METHODS

Starting in 1974, collections of *P. coronata*, each collection consisting of a few infected leaves from the same or adjacent plants, were made annually in southern Texas from susceptible oat cultivars late in the growing season, usually in March or April. The collections were mailed to Iowa where they were increased in bulk in May or June and stored on the host leaves in a refrigerator at 4 C over the summer and early fall. In the late fall, spores from a single well-isolated uredium from each collection were increased on the susceptible oat cultivar Markton to produce as much inoculum as needed. To minimize contamination, spores were transferred in a chamber having positive pressure, inoculating needles were sterilized between isolates with flaming alcohol, and infected plants were held in individual isolation chambers consisting of glass or plastic cylinders plugged at the top with cotton.

Plants of oat cultivars carrying known genes for resistance to *P. coronata*, including TAM 0-301 and TAM 0-312 (7), were inoculated as soon as the first seedling leaf was fully expanded by applying urediospores suspended in light oil to the plants with an atomizer, at a concentration of spores sufficient to ensure an infection severity of 50-100 uredia per leaf. Following application of spores, plants were held in a dew chamber without light for 16 hr at 20 C.

Except where noted, plants were held for incubation on greenhouse benches at a temperature of 20 C. The work was done during the fall and winter when outside temperatures were cold and it was possible to maintain a uniform temperature by a combination of forced-air heating and ventilation. Supplementary light was provided to give a total of 14 hr per day of light at an intensity that resulted in vigorous host growth and normal development of the fungus on susceptible control plants. Where temperature was critical, plants were incubated in growth chambers set for a 14-hr daylength. Temperatures and incubation periods for growth chamber trials are shown in Table 1.

All host plants were grown in a greenhouse soil mix that had

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sufficient fertility to maintain vigorous host growth until the material was scored. Plants held in the greenhouse were scored 12 days after inoculation according to the scale shown in Table 2. Plants held in the growth chambers were scored following the incubation periods and according to the scale shown in Table 1. To assure consistency of results, all scoring was done by the same experienced plant pathologist.

All F<sub>2</sub> populations used in genetic studies were handled as above and the plants were incubated in growth chambers. Plants

TABLE 1. Reaction types<sup>a</sup> of oat cultivar TAM 0-301 to 14 isolates<sup>b</sup> of *Puccinia coronata* at three temperatures

Isolate or race no.	Temperature and incubation period					
	20 C		23 C		26 C	14 days at 20 C
	14 days	19 days	12 days	16 days	10 days	4 days at 26 C
264B	0	0	0	0	0	0
20-10-1	0	2	1-	3-	4	4
81-7-1	0	0	0	0	0	0
111-7-1	0	1-	1-	3+	4	4-
112-10-1	0	2-	...	...	4	4
113-10-1	0	1-	1-	4-	4	4
115-10-1	1	2+	...	...	4	4
116-10-1	1-	2-	1-	3	4	4-
117-10-1	0	0	0	1-	3+	3+
118-10-1	4	4	...	...	4	4
122-10-1	3+	4	4-	4	4	4
123-10-1	1-	2-	2	4-	4	4
125-10-1	1+	2+	3	4	4	4
C-1-2-10-1	0	1-	0	3-	4	4-

<sup>a</sup>Symbols: 0 = necrotic lesions without uredia; 1 = necrotic lesions, some of which contain small uredia; 2 = necrotic lesions containing small to mid-sized uredia; 3 = strongly chlorotic areas containing mid-sized to large uredia; 4 = faintly chlorotic areas containing large uredia. Plus and minus signs indicate, respectively, a somewhat greater or lesser degree of uredial development; ... indicates that no data were obtained.

<sup>b</sup>Except for the control race 264B, isolates were from collections made in 1980.

TABLE 2. Numbers of isolates of *Puccinia coronata* from Texas tested for virulence and percentages of isolates inducing different reaction types in oat cultivars TAM 0-301 and TAM 0-312 from 1974 to 1980

Year	Isolates (no.)	TAM 0-301				TAM 0-312				
		Percent isolates in reaction type <sup>a</sup> :				Percent isolates in reaction type <sup>a</sup> :				
		1.0	1	2	3,4	(no.)	1.0	1	2	3,4
1974	79	100	0	0	0	43	86	12	2	0
1975	77	94	5	1	0	0 <sup>b</sup>	...	...	...	...
1976	106	95	3	2	0	106	93	0	1	6
1977	116	90	4	4	2	111	87	1	2	10
1978	97	70	8	15	6	100	64	1	0	35
1979	97	46	10	37	6	108	41	0	4	56
1980	161	17	24	40	19	180	14	1	1	83

<sup>a</sup>1.0 = no uredia; 1 = necrotic lesions, some of which contain small uredia; 2 = necrotic lesions containing small- to mid-sized uredia; 3,4 = mid- to full-sized uredia sometimes in chlorotic areas, no necrosis.

<sup>b</sup>TAM 0-312 was not tested in 1975 because of uncertainty regarding its future importance.

representing both parents were always included as controls to provide a check on contamination and other potential problems.

Since there is not complete agreement among plant pathologists on the precise meaning of the term virulence, we are defining it here as "degree of pathogenicity of the fungus."

## RESULTS

**Greenhouse trials.** The cultivar TAM 0-301 was highly resistant to all of the 79 isolates of *P. coronata* that originated in southern Texas in 1974 (Table 2). In 1975 and 1976, a few isolates induced type "1" or "2" reactions. The percentage of such isolates gradually increased during the next 3 yr, and in 1980 they comprised about one-fifth of the total. Avirulent isolates showed a corresponding decrease in prevalence, and they comprised less than one-fifth of the total in 1980.

The isolates showed a much different pattern of virulence on TAM 0-312 during the years it was tested. In 1974, none of the isolates induced a susceptible reaction in TAM 0-312 but several showed a small amount of sporulation. This cultivar was not tested in 1975, but the percentage of isolates rated as virulent on it increased rapidly from 1976 to 1979, with such isolates comprising 83% of the total in 1980. During this period only a few isolates induced the intermediate reaction types "1" and "2" on TAM 0-312, a pattern much different from that observed for TAM 0-301. Except for the small number of intermediate isolates, these data could be explained by assuming a major mutation from avirulence to virulence with subsequent strong selection pressure favoring the mutant.

**Effects of temperature.** To investigate possible effects of temperature, plants of TAM 0-301 were inoculated with many isolates of *P. coronata* having various degrees of virulence under greenhouse conditions, and held in growth chambers at three mean temperatures (Table 1). The isolates shown in Table 1 are representative of the larger number that were tested and were chosen to show the range of reaction types that occurred at the different temperatures. Some, such as 81-7-1, were avirulent at low, moderate, and high temperatures, and, based on degrees of virulence toward 22 lines of oats carrying different resistance genes, evidently corresponded to forms of the fungus occurring before the release of TAM 0-301, such as the old isolate of race 264B that was used as a control. A complete range of reaction types from no sporulation through low and intermediate degrees of sporulation to full sporulation occurred when plants were held at 20 C for 14 days. After five more days at 20 C, some isolates induced somewhat more susceptible reactions, but there was still a complete range of reactions from isolates that produced no spores to those that displayed full virulence. Isolates held at 23 C for 12 days showed a similar pattern, except that many were somewhat more virulent than they had been at 20 C. Plants inoculated with isolates that induced any sporulation at 12 days were appreciably more susceptible 4 days later, with changes from a resistant "1" or "2" type reaction to a susceptible "3" or "4" type reaction being common. Uredia on susceptible control cultivars were fully developed at the end of 10 days on plants held at 26 C. At this temperature almost all isolates that had induced even slight sporulation at 20 or 23 C were fully virulent. The old isolate of race 264B and isolate 81-7-1, however, remained avirulent on TAM 0-301 at 26 C.

Infected plants that had been held at 20 C for 14 days were

TABLE 3. Segregation of F<sub>2</sub> populations from the cross Richland × TAM 0-301 for reaction to isolates of *Puccinia coronata*

Pop. no.	Isolate		Temp. (C)	Reaction types 0 and 1 <sup>a</sup>	Reaction type 2 <sup>a</sup>	Reaction type 4 <sup>a</sup>	Ratio tested	χ <sup>2</sup>	P
	First inoc.	Second inoc.							
1	111-7-1	None	23		82	17	3:1	3.24	0.10-0.05
2	116-10-1	None	23		76	26	3:1	0.013	>0.90
3	81-7-1	111-7-1	20	156		42	3:1	1.52	0.25-0.10
4	81-7-1	116-10-1	20	146		51	3:1	0.83	0.90-0.75

<sup>a</sup>See footnote to Table 1 for descriptions of reaction types.

transferred to a 26 C chamber. At the end of 4 days in the 26 C chamber, all but two of the isolates tested induced virulent reaction types that were virtually indistinguishable from those on plants that had been held at 26 C for the entire incubation period.

**Distribution of virulence genes in the fungus population.** Isolates that were virulent or moderately virulent on TAM 0-301 in the greenhouse were relatively uniform for virulence pattern on the other 23 differential cultivars that were tested. In 1980, for example, about 95% of such isolates showed the same pattern of virulence (ie, they were all virulent on the same four differentials and were all avirulent on the same 19 differentials). This pattern (or "race") was the one that was most common in 1980 (7). In contrast, isolates that were avirulent on TAM 0-301 included a variety of phenotypes for virulence pattern on other differentials, with the most common phenotype accounting for only about half of the total.

**Inheritance.** Genetic studies were carried out by classifying the reaction types of F<sub>2</sub> seedling plants from crosses between the susceptible cultivar Richland and TAM 0-301. Plants of F<sub>2</sub> populations tested for reaction to isolates 111-7-1 and 116-10-1 (Table 1) were held at 23 C until TAM 0-301 control plants showed some sporulation. The F<sub>2</sub> plants then fell into typical 3:1 ratios of intermediate to susceptible reaction types (Table 3), indicating that a single dominant gene conditioned reaction to these isolates.

Trials were then carried out to determine the inheritance of the high resistance of TAM 0-301 to isolate 81-7-1, and whether the same gene or genes conditioned the intermediate reaction to isolates 111-7-1 and 116-10-1. Plants of F<sub>2</sub> populations in the first-leaf stage were inoculated with isolate 81-7-1, and then inoculated with isolate 111-7-1 four days later after the appearance of the second leaf, but before sporulation had started on the first leaf. The plants were held at 20 C where a resistant reaction to isolate 111-7-1 would be expressed. Nine days after the initial inoculation, the first leaves of the plants were examined for reaction to isolate 81-7-1. The ratios of resistant to susceptible plants indicated single gene inheritance. This gene was presumably the same as that designated previously as *Pc-58* (10). Plants were marked as susceptible or resistant, and a few days later all plants that had been classified as susceptible to isolate 81-7-1 on the basis of the reaction of the first leaf also showed susceptibility of the second leaf to isolate 111-7-1. The second leaves of all plants having first leaves rated as resistant to isolate 81-7-1 were resistant to isolate 111-7-1. This test was repeated by using isolate 116-10-1 in place of 111-7-1 with the same results. It was concluded that the same single gene that conditioned the high type of resistance to 81-7-1 also conditioned resistance to isolates 111-7-1 and 116-10-1.

## DISCUSSION

The process responsible for the gradually increasing virulence toward TAM 0-301 reported here is of interest. It is unlikely that this virulence resulted directly from recombination on *Rhannus* because isolates of *P. coronata* originating on *Rhannus* during this period did not show any increase in virulence on TAM 0-301 (7). Outside the context of genetic recombination, however, a sequence of steps leading to the appearance of isolates fully virulent on TAM 0-301 can be hypothesized. The first step, assuming we are concerned with a single virulence gene in the pathogen (2), could be a mutation (or possibly some form of intragenic recombination) in fungus strains heterozygous at the *Pc-58* locus toward a degree of virulence permitting limited sporulation at high, but not low, temperatures. From this base, subsequent mutations could be toward greater sporulation at high temperatures, followed finally by sporulation at low temperatures.

In a recent review of the limited literature on progressive increases in virulence of the cereal rust fungi, McIntosh and Watson (5) stated that the genetic nature of the variation is unknown and suggested that it could be either the result of changes at modifying loci or of variation within major pathogenicity loci. We believe that our work, which shows that the same gene conditions reaction to isolates that are avirulent and to those that are moderately virulent or temperature sensitive, favors the latter hypothesis.

The possibility of a more complex genetic explanation should not be discounted. An example of possible pitfalls in such genetic speculation is furnished by the report of Zimmer and Schafer (12) regarding a small increase in virulence of race 202 of *P. coronata* on the oat cultivar Ascençao (12). Another investigation (8) showed that the cultivar Ascençao carried two genes for resistance, one of which conditioned high resistance and was epistatic to the second, which conditioned a lesser degree of resistance. Thus, the supposed small mutation of the fungus toward greater virulence also could be interpreted as a major mutation for virulence toward the host gene conditioning the high resistance.

It is tempting to speculate that the small stepwise mutations hypothesized here occur commonly and that even a moderately virulent mutant has a selective advantage resulting in an increase in its frequency, which forms a base for the next mutational increase. If this were the case, the frequency of other virulence genes in mutant isolates would be expected to correspond to the frequency of those virulence genes in the "wild type" fungus population. Our data, however, showed that isolates virulent or moderately virulent on TAM 0-301 were not thus randomly distributed, suggesting that the original mutations were relatively rare events.

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